REMARKS

Claims 1-33 and 43-71 are pending in this application, and claims 1-15 and 58-71 were subject to examination in the Office Action dated June 12, 2009. Claims 1, 3-4, 10, 14, 58-60, 62, 64-65, 67 and 69 have been amended herein, and claims 16-22, 27-32 and 43-57 have been canceled as directed to non-elected inventions and to reduce excess claim fees without disclaimer of any subject matter or prejudice to the filing of a divisional application directed thereto. New claim 72 has been added herein. New claim 72 is a dependent claim and recites "wherein the phospholipid consists essentially of phospholipids acylated by C4 to C12 fatty acids." Support for this claim language is found in the language of the original claims and throughout the specification. It is believed that no new matter is added by these amendments and new claim and their entry and consideration are respectfully requested. In light of the amendments to the claim set, Applicants respectfully request reconsideration of this application and allowance of the pending claims to issue.

Claim Objections

The Office Action states that claims 60 and 65 are objected to based on the recitation of "C4 to C12 fatty acids." The specification states that "the soluble phospholipid comprises, consists essentially of, or consists of acylated C4 to C10 or C12 fatty acids." Specification page 14, lines 15-16. Applicants respectfully submit that the cited language means that the soluble phospholipids may comprise acylated C4 to C10 fatty acids or acylated C4 to C12 fatty acids; C11 is not excluded. Such meaning would be clear to one of ordinary skill in the art. Accordingly, Applicants respectfully request withdrawal of this objection.

35 U.S.C. § 112

Claims 1-15 and 58-71 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Reconsideration is respectfully requested in view of the amendments to the claims and remarks presented below.

A. The Office Action states that claims 1, 58, 62 and 67 are allegedly indefinite because it is unclear if the "sample" of the preamble is the "sample" of step (a). Claims 1, 58, 62 and 67 are amended herein to recite "a blood or plasma sample from a subject, the

method comprising: (a) creating a mixture by combining *in vitro* the[[a]] blood or plasma sample." These amendments merely clarify the claimed subject matter and are not narrowing in effect.

B. The Office Action states that in claim 1, step (c) the activity of Factor X_a or thrombin is allegedly unclear and that claims 3-4, 58, 62 and 67 suffer similar deficiencies. Claims 1, 58, 62 and 67 have been amended herein to recite in step (c) "detecting Factor X_a or thrombin enzyme activity, wherein the enzyme activity of Factor X_a or thrombin correlates with clotting factor activity in the sample, thereby evaluating clotting activity in the sample." Claim 3 has been amended herein to recite "wherein the level of thrombin enzyme activity correlates with Activated Protein C resistance in the sample" and claim 4 has been amended herein to recite "wherein the level of thrombin enzyme activity inversely correlates with Protein S levels in the sample." Similarly, Claim 14 has been amended herein to recite "[t]he method of Claim 1, further comprising comparing the detected thrombin enzymatic activity with a standard."

C. The Office Action further states that claims 1, 58, 62 and 67 allegedly lack sufficient antecedent basis for the limitation in step (b) of "the mixture of (a)." Claims 1, 58, 62 and 67 have been amended herein to recite in step (a) "creating a mixture by combining *in vitro* the[[a]] blood or plasma sample from the[[a]] subject with ...."

D. The Office Action states that the term "Protein C resistance" is allegedly unclear in claim 3 because it is unclear as to what objects Activated Protein C (APC) has resistance. Applicants respectfully submit that Activated Protein C resistance is a well-known hemostatic disorder characterized by a reduced response to APC (see, e.g., Activated Protein C resistance entry from Wikipedia). Additionally, one of ordinary skill in the art would understand the term "Protein C resistance" when read in light of the specification. The specification states that "[p]rotein C prevents uncontrolled coagulation and migration of the activated blood clotting factors from the site of vascular injury." Specification at page 24, lines 1-2. Furthermore, the specification states that "[a]ddition of APC to plasma or blood from normal subjects results in a slowing down of the coagulation process and a prolonged clotting time (alternative results include reduced thrombin enzymatic activity or reduced FX_a

activity, *etc.*). APC resistance is manifested as an impairment in this inhibitory pathway, with continued rapid clotting (or alternatively, high thrombin enzymatic activity, high FX_a activity, *etc.*)." Specification at page 24, lines 16-21. Thus, Applicants respectfully submit that the term "Protein C resistance" would be sufficiently clear to one of ordinary skill in the art in view of the knowledge in the art regarding APC resistance and the guidance in the specification.

Having addressed the Examiner's rejections raised in the Office Action with respect to indefiniteness, Applicants respectfully request that the rejections of claims 1-15 and 58-71 under 35 U.S.C. § 112, second paragraph, be withdrawn.

35 U.S.C. § 102(b)

The claims are subject to two rejections on the basis of lack of novelty under 35 U.S.C. § 102(b), each of which will be addressed individually below.

1. Gempeler et al.

Claims 1, 5-9 and 13 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Gempeler et al. as evidenced by Hürter et al. and Boos et al. The Office Action states:

> Gempeler anticipates the claims by teaching a body fluid coagulation-potential assay comprising a body fluid, including plasma; contacting with phospholipids, calcium ($CaCl_2$), and an activator (e.g. RVV-V).... Although Gempeler does not teach the presence/degree thereof of a particular phospholipid, as evidenced by Hürter, plasma intrinsically comprises a degree of phospholipids, including phosphatidylserine (PS) and phosphatidylethanolamine (PE) among others (Hürter, table II). Although Gempeler or Hürter do not explicitly teach that plasma contains "soluble" phospholipids or phospholipids that are soluble in a blood or plasma sample, plasma contains soluble phospholipids, as evidenced by Boos which teaches that "the lipids of the blood plasma (cholesterol, triglycerides and phospholipids) are by their nature water-insoluble and are present in soluble form in the aqueous medium of the blood due to the combination with specific proteins." (page 2, paragraph [0027]).

(Office Action, paragraph spanning pages 4-5; *emphasis added*).

To anticipate a claim, each and every element of the claim must be taught, either expressly or inherently, in a single prior art reference. *See e.g.*, *Verdegaal Bros. v. union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987) ("a claim is

anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference"). Applicants respectfully submit that the outstanding rejection fails to satisfy the legal requirements for anticipation as each and every element of the claimed invention is not disclosed by the Gempeler et al. reference.

Gempeler et al. only teaches the use of conventional, insoluble (i.e., aggregated, solid-phase) phospholipids that are prepared from natural sources, such as rat brain cephalin, rather than phospholipids that are soluble in the sample as recited by the present claims, which as described in the specification at page 13, lines 26-27 comprise "essentially no aggregates (e.g. as lamellar or non-lamellar structures)." Gempeler et al. is silent regarding soluble phospholipids as recited by the present claims.

As discussed during the telephonic interview, Gempeler et al. is limited to the use of conventional, insoluble phospholipids as specifically shown in Figure 4 of the Gempeler et al. reference, which demonstrates a bilayer membrane formed by the phospholipids and is described as "a diagram illustrating the principle of the present assay." Gempeler et al. at page 13, line 30 and page 15, line 1. In addition, Gempeler et al. states "[r]ecalcification of citrated blood or plasma is employed to complete the assembly of the prothrombinase complex on the phospholipid surface and clotting begins." Gempeler et al. at page 15, lines 5-7, *emphasis added.* Thus, the phospholipids disclosed in Gempeler et al. are insoluble phospholipids that form membrane-like structures.

Furthermore, the Office Action states that Hürter et al. teaches that plasma intrinsically comprises a degree of phospholipids, including phosphatidylserine (PS) and phosphatidylethanolamine (PE) and erroneously relies on Boos et al. to argue that these phospholipids are soluble.

However, the phospholipids disclosed in Hürter et al. and Boos et al. are from natural sources, such as erythrocytes and blood plasma. As discussed in Applicant's previous response of January 23, 2009, naturally occurring sources of phospholipids will primarily have fatty acids with hydrocarbon tails that are C16 or longer and will form insoluble membrane structures in aqueous solutions. It is the chain length of the fatty acid tail that primarily determines solubility, not the head group (e.g., PS or PE).

The reliance upon Boos et al. to teach the solubility of these natural phospholipids is erroneous because as noted in the Office Action these naturally insoluble phospholipids "are present in soluble form in the aqueous medium of the blood due to the combination with specific proteins." Boos et al at page 2, paragraph [0027]. In the next sentence, Boos et al. continues by stating that "[t]hese particulate complexes are designated as plasma lipoproteins." Boos et al at page 2, paragraph [0027]. Applicants submit that those skilled in the art often refer to lipoproteins as "solubilized" in blood or plasma because they form a stable particulate suspension (i.e., do not settle out). Lipoproteins are clearly particulate and act as protein-based micelles. In fact, lipoproteins are named based on how they are purified by ultracentrifugation (high density, low density, etc.) (see, for example, Boos et al. page 2, paragraph [0028]). Thus, lipoproteins are not soluble in plasma, and Boos et al. does not teach that plasma comprises soluble phospholipids.

The Office Action further states that the plasma sample of Gempeler et al. intrinsically comprises soluble phospholipids and that the claims do not require the phospholipid to be added to the sample. However, Applicants respectfully submit that the claims do require the soluble phospholipid to be added to the sample. Nonetheless, independent claims 1, 58, 62 and 67 are amended herein to recite in step (a) "creating a mixture by combining *in vitro* the[[a]] blood or plasma sample from the[[a]] subject with: (i) a phospholipid that is soluble in the sample ..." to further clarify the claimed methods. Even if, for the sake of argument, plasma did intrinsically comprise soluble phospholipids, the claim language requires the soluble phospholipid to be combined with (i.e., added to) the sample *in vitro*. Gempeler et al. does not disclose the combination of a blood or plasma sample with a phospholipid soluble in the blood or plasma sample *in vitro* to create a mixture. Thus, Applicants respectfully submit that Gempeler et al. can be further distinguished from the present invention on this basis.

Finally, as discussed during the telephonic interview held September 29, 2009, independent claims 1, 58 and 62 are amended herein to recite "a phospholipid that is soluble in the sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 fatty acids" to further distinguish the claimed subject matter from the cited references. Support for this amendment is found in the language of original claim 60 and throughout the specification at least, for example, on page 14, lines 14-16. Independent claim 67 recites

similar language ("a phospholipid that is soluble in the sample and consists essentially of phospholipids acylated by C2 to C14 fatty acids").

In view of the foregoing, Applicants respectfully submit that Gempeler et al. as evidenced by Hürter et al. and Boos et al. fails to disclose or suggest the claimed methods using a phospholipid that is soluble in the sample, more specifically, a soluble phospholipid acylated by C4 to C12 (or C2 to C14) fatty acids. As such, the rejection over Gempeler et al. is legally deficient and cannot be maintained. Thus, Applicants respectfully request that this rejection be withdrawn.

2. Triplett et al.

Claims 1, 2, 5, 7 and 8 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Triplett et al. as evidenced by Hürter et al. and Boos et al. The Office Action states that "Triplett teaches combining a plasma sample; soluble phospholipids, including phosphatidylserine and phosphatidylethanolamine obtainable by extraction or commercially available; a contact activator; and calcium (chloride), including the incubation thereof to activate thrombin/detect thrombin activity (abstract; summary; column 5, paragraph 3, 4; examples 4 and 5)." Office Action page 5.

Triplett et al. only discloses the use of conventional, insoluble (i.e., aggregated, solid-phase) phospholipids that are prepared from natural sources. Specifically, Triplett et al. states that "[a] clotting test, sensitive to LA can be carried out by mixing a plasma sample with a suitable amount of a phospholipid suspension . . .." Triplett et al. at page 4, lines 23-25, *emphasis added*. Applicants respectfully note that a "suspension" is "a dispersion of fine solid particles in a liquid or gas, removable by filtration" (Encarta dictionary). Thus, the "phospholipid suspension" of Triplett et al. is composed of insoluble phospholipid particles. Additionally, in Examples 4 and 5 Triplett et al. discusses the use of phospholipids prepared, as described in Example 2, from rabbit brain kephalin. Again, as discussed above phospholipids from natural sources, such as animals, primarily have fatty acids that are C16 or longer and spontaneously form micelles or other membrane-like structures in aqueous solutions such as blood or plasma samples. Therefore, the phospholipids

disclosed in Triplett et al. are all conventional insoluble preparations isolated from natural sources, which primarily have fatty acids that are C16 or longer.

Applicant's respectfully submit that the Office Action's reliance on Hürter et al. and Boos et al. is misplaced since Boos et al. is disclosing lipoproteins. As discussed above with respect to the Gempeler et al. reference, lipoproteins are particulate; therefore, Hürter et al. and Boos et al. do not teach or suggest that plasma comprises soluble phospholipids. Thus, Applicants respectfully submit that Triplett et al. fails to disclose a phospholipid soluble in a blood or plasma sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 (or C2 to C14) fatty acids as recited by the present claims. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Further, Applicants reiterate that claims 1, 58 and 62 are amended herein to recite "a phospholipid that is soluble in the sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 fatty acids" to further distinguish the claimed subject matter from the cited references. Claim 67 recites similar language ("a phospholipid that is soluble in the sample and consists essentially of phospholipids acylated by C2 to C14 fatty acids").

Applicants having clarified that neither Gempeler et al. nor Triplett et al. disclose phospholipids that are soluble in a blood or plasma sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 (or C2 to C14) fatty acids respectfully request that the rejection of claims 1, 5-9 and 13 and claims 1, 2, 5, 7 and 8 under 35 U.S.C. § 102(b) be withdrawn.

35 U.S.C. § 103(a)

The Office Action raises a large number of rejections under 35 U.S.C. § 103(a), which can be classified into three groups corresponding to the primary reference cited. The primary references are combined with a variety of secondary references in different combinations to generate numerous rejections under 35 U.S.C. §103(a). Each of these rejections is addressed individually below.

1. Gempeler et al.

Claims 1-3, 5-9, 13 and 14 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Gempeler et al. in view of Hürter et al. and Boos et al. Each of these

references has been discussed above with respect to the rejections under 35 U.S.C. §102(b). Applicants respectfully submit that none of these references teaches or suggests the use of a phospholipid that is soluble in a blood or plasma sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 (or C2 to C14) fatty acids. Accordingly, Applicants respectfully submit that the subject matter of claims 1-3, 5-9, 13 and 14 is nonobvious over the combination of Gempeler et al., Hürter et al. and Boos et al.

The following claims stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Gempeler et al. in view of Hürter et al. and Boos et al. and further in view of one or more additional secondary references:

- Claim 4 further in view of Matschiner;
- Claims 9 and 15 further in view of Gorman et al. and Zhai et al.;
- Claim 15 further in view of Opalsky et al.;
- Claims 11, 12, 58, 59 and 61 further in view of Sigma;
- Claims 10, 60, 62, 65, 67 and 70 further in view of Majumder et al.; and
- Claims 10, 60 and 62-71 further in view of Sigma and Majumder et al.

Applicants respectfully submit that the teachings of these additional secondary references fail to remedy the deficiencies of the Gempeler et al., Hürter et al. and Boos et al. references discussed above. Specifically, none of the cited references, taken alone or in any combination, discloses or suggests a method of evaluating clotting activity in a blood or plasma sample from a subject comprising creating a mixture by combining *in vitro* the sample with a phospholipid that is soluble in the sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 (or C2 to C14) fatty acids. Thus, the subject matter of the above referenced claims is nonobvious over the combination of the above cited references.

2. Triplett et al.

Claims 1-3, 5, 7-9 and 12-15 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Triplett et al. in view of Hürter et al. and Boos et al. Each of these references has been discussed with respect to the rejections under 35 U.S.C. §102(b). Applicants respectfully submit that none of these references teaches or suggests the use of a phospholipid that is soluble in a blood or plasma sample, wherein the phospholipid comprises

phospholipids acylated by C4 to C12 (or C2 to C14) fatty acids. Accordingly, Applicants respectfully submit that the subject matter of claims 1-3, 5, 7-9, and 12-15 is nonobvious over the combination of Triplett et al., Hürter et al. and Boos et al.

The following claims stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Triplett et al. in view of Hürter et al. and Boos et al. and further in view of one or more additional secondary references:

- Claim 4 further in view of Matschiner;
- Claims 9 and 15 further in view of Gorman et al. and Zhai et al.;
- Claim 15 further in view of Opalsky et al.;
- Claims 10, 62, 65, 67 and 70 further in view of Majumder et al.;
- Claims 11, 58, 59 and 61 further in view of Sigma; and
- Claims 10, 60 and 62-71 further in view of Sigma and Majumder et al.

As discussed above, none the cited references, taken alone or in any combination, disclose or suggest a method of evaluating clotting activity in a blood or plasma sample from a subject comprising creating a mixture by combining *in vitro* the sample with a phospholipid that is soluble in the sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 (or C2 to C14) fatty acids. Thus, Applicants respectfully submit that the subject matter of the above referenced claims is nonobvious over the combination of Triplett et al. and the other cited references.

3. Tans et al.

Claims 1, 3-5, 7, 9, 11-15, 58, 62 and 63 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Tans et al. The Office Action states "[a]lthough Tans does not explicitly recite a soluble phospholipid, the selection of a soluble phospholipid in the sample would have been a routine matter of optimization on the part of the artisan of ordinary skill." Office Action page 26.

Tans et al. only discusses the use of insoluble phospholipids in clotting assays. This is demonstrated throughout Tans et al. in their reference to "phospholipid vesicles," which are insoluble phospholipids. Tans et al. Abstract and Methods section page 21865. Furthermore, in the description of the lipid preparations in the methods section, Tans et al. describes the use of rabbit brain cephalin, an insoluble phospholipid preparation. Tans et al.

further states that the phospholipid vesicles were prepared as described earlier in Rosing et al. Tans et al. page 21865 reference (4) listed on page 21872. In the experimental procedures section on page 275, Rosing et al. describes the preparation of phospholipid vesicles and recites the chain length of the phospholipids as C18.

Additionally, in the introduction section on page 274, Rosing et al. states that "[i]t is now generally accepted that Ca^{+}, a phospholipid surface, and factor V_a are required for prothrombin activation under physiological conditions. It has been shown that the above mentioned clotting factors have to be absorbed on the phospholipid bilayer surface in order to acquire efficient interaction" (*emphasis added*). Tans et al. demonstrates this understanding by its utilization of insoluble phospholipid vesicles to quantitate meizothrombin and thrombin formation in their coagulation experiments. Furthermore, Tans et al. states on page 21867 "[i]t is well known that the lipid composition of procoagulant membranes may strongly influence their ability to accelerate coagulation factor activation. ... we have measured the influence of variation of the amount of phosphatidylserine, present in procoagulant membranes ..." (*emphasis added*). In contrast, as stated in the present specification the inventors have made an "unexpected discovery that solubilized phospholipids are functionally equivalent to platelet membranes and can substitute them in membrane-catalyzed reactions within the intrinsic pathway." Specification page 11, lines 21-24, *emphasis added.* Furthermore, the specification states that "[t]he current understanding of the intrinsic pathway is that a two-dimensional membrane surface is required to bring the individual factors together and to accelerate complex formation." Specification page 11, line 33 and page 12, lines 1-2.

Therefore, Applicants respectfully submit that Tans et al. fails to teach or suggest phospholipids that are soluble in a blood or plasma sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 (or C2 to C14) fatty acids. Accordingly, Applicants respectfully submit that the subject matter of claims 1, 3-5, 7, 9, 11-15, 58, 62 and 63 is nonobvious over Tans et al. and respectfully request that the rejection under 35 U.S.C. § 103(a) over this reference be withdrawn.

Further, Applicants again note that claims 1, 58 and 62 are amended to herein recite "a phospholipid that is soluble in the sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 fatty acids" to further distinguish the claimed subject matter from the cited references. Independent claim 67 recites similar language ("a

phospholipid that is soluble in the sample and consists essentially of phospholipids acylated by C2 to C14 fatty acids").

The following claims stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Tans et al. in view of one or more additional secondary references:

- Claims 10, 60, 65 and 70 in view of Majumder et al.;
- Claims 9 and 15 in view of Gorman et al. and Zhai et al.; and
- Claim 15 in view of Opalsky et al.

However, these secondary references fail to remedy the deficiencies of the Tans et al. reference discussed above. Specifically, none of the cited references taken alone or in any combination teach or suggest a method of evaluating clotting activity in a blood or plasma sample from a subject comprising creating a mixture by combining *in vitro* the sample with a phospholipid that is soluble in the sample, wherein the phospholipid comprises phospholipids acylated by C4 to C12 (or C2 to C14) fatty acids.

Finally, the Office Action appears to suggest that it would have been a matter of routine optimization to select the fatty acid chain lengths and/or phospholipid concentrations recited by some of the claims. Applicants respectfully disagree. MPEP 2144.05 (Obviousness of Ranges) states that only the optimization of "result-effective variables" can be considered routine. Specifically, this section states "[a] particular parameter must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation." In the present case, the prior art did not recognize that soluble phospholipids can be used in place of conventional insoluble phospholipids, or the advantages to be achieved thereby. Thus, phospholipid solubility as well as phospholipid concentrations and fatty acid chain lengths that are compatible with solubility of the phospholipids are not result-effective variables, and it would not have been obvious or routine for the ordinarily skilled worker at the time of invention to optimize these parameters.

Having addressed the Examiner's rejections raised in the Office Action with respect to obviousness, Applicants respectfully request that the rejection of the above mentioned claims under 35 U.S.C. § 103(a) be withdrawn.

New Claim

New claim 72 is added herein and recites "[t]he method of Claim 1, wherein the phospholipid consists essentially of phospholipids acylated by C4 to C12 fatty acids." Support for this new claim is found in the language of original claim 70 and in the specification, at least for example, on page 14, lines 15-16. New claim 72 is believed to be free of all the rejections cited above for pending claims 1-33 and 43-71 for all of the reasons articulated herein in support of these pending claims. Thus, entry and allowance of this new claim is respectfully requested.

Conclusion

Applicants submit that the points and concerns raised by the Examiner in the outstanding Office Action have been addressed in full. Therefore, it is respectfully asserted that this application is in condition for allowance, which action is respectfully requested. Should the Examiner have any remaining concerns, it is respectfully requested that he contact the undersigned attorney at (919)-854-1400 to expedite the prosecution of this application to allowance.

Respectfully submitted,

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Enclosure: Wikipedia entry for Activated Protein C resistance

CERTIFICATION OF TRANSMISSION

I hereby certify that this correspondence is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4) to the U.S. Patent and Trademark Office on October 13, 2009.

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